mutants, *akirin* mutant embryos have misattached or missing muscles and severely altered muscle morphology. *Akirin* interacts genetically and physically with Twist and is localized to Twist-dependent enhancers *in vivo*. Accordingly, Twist target gene expression is highly reduced in *akirin* mutants. While Akirin has been identified as a component of other transcriptional pathways, its mode of action in these pathways remains unclear. We determined that *akirin* colocalizes and genetically interacts with subunits of the Brahma SWI/SNF-class chromatin remodeling complex at Twist-target genes. This suggests that *akirin* mediates a novel link between Twist and chromatin remodeling complexes to facilitate Twist-regulated transcription during *Drosophila* myogenesis. These results also provide a common mechanism by which *akirin* regulates the activities of multiple TFs during development, the immune response and homeostasis.

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Program/Abstract # 263
The Drosophila estrogen-related receptor is required for the transition from embryonic to larval metabolism
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Drosophila larval metabolism is exquisitely tuned to promote exponential growth. This growth state is in contrast with embryonic metabolism, which is dependent on intrinsic energy reserves. Despite these differences, little is known about how a developing animal transitions between embryonic and larval metabolic states. We have discovered that the *Drosophila* ortholog of the vertebrate estrogen-related receptor, dERR, directs this developmentally-regulated metabolic switch. dERR null mutants die as second instar larvae with abnormally low ATP levels, diminished triglyceride stores, elevated levels of circulating sugars, and decreased concentrations of lactate, α-ketoglutarate, and malate. These defects can be attributed to reduced expression of key genes in several metabolic pathways, including all of the genes that act in glycolysis. dERR appears to directly regulate these pathways as there are putative dERR binding sites in nearly all of the misregulated genes examined. Intriguingly, the metabolic pathways induced by dERR at the onset of larval development are similar to the Warburg effect, by which cancer cells use glucose to support biomass production and rapid proliferation. Our results demonstrate that the Warburg effect can be utilized in the context of normal developmental growth, indicate that dERR establishes a metabolic state that supports larval development, and implicate the ERR receptor family as central regulators of the metabolic parameters that support cancer. This work was supported by the NIH (1R01DK075607). JMT was supported by the NIDDK (F32DK083864).

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Program/Abstract # 264
Novel animal model for studying the roles of the upstream open-reading frames
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Regulation of gene expression may be achieved at multiple levels. Among the regulatory mechanisms, translational control is an immediate early response that becomes crucial in the absence of transcription. It is now known that the upstream open reading frames occur in approximately 10%–25% of 5′-UTRs and have been generally found to repress translation of the downstream open reading frame. However, these upstream open reading frames mediated translational inhibition has less been studied *in vivo*. In this report, we developed an *in vivo* system to study the upstream open reading frame mediated translational inhibition by using model animal, zebrafish (*Danio rerio*). We generated a transgenic line *Tg*(*CMV*:*uGFP*), in which the upstream open reading frame from human CCAAT/enhancer-binding protein homologous protein is fused with GFP and driven by cytomegarovirus (CMV) promoter. We found that the GFP signal was not apparent under normal condition, although the *gfp* mRNA was transcribed throughout the embryos. These indicate that the translation of *gfp* is completely inhibited by the upstream open reading frame cassette. Interestingly, when *Tg*(*CMV*:*uGFP*) embryos were treated with thapsigargin, the GFP was greatly detected in the brain, indicating that environmental stimulus may direct the upstream open reading frame cassette to abolish its translational inhibition. Thus, zebrafish is an excellent animal model for studying the upstream open reading frame mediated translational inhibition.

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Program/Abstract # 265
The molecular structures and expression patterns of two distinct zebrafish Dickkopf 3 genes
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The Wnt signaling pathway is a cellular communication pathway that plays critical roles in development and disease. A major class of Wnt signaling regulators is the Dickkopf (Dkk) family, which is a secreted glycoprotein. The DKK family has been identified in birds and mammals, and has been known that it consists of *dkk1*, *2*, *3*, *4* and a *dkk3*-related gene (*soggy*). However, in low vertebrates, only *dkk1* has been defined, the others are still unknown. Here, we cloned two zebrafish *dkk3* genes, which were *dkk3* and *dkk3*-related genes *(dkk3r*, also named the long-isoform *dkk3*). Based on the unrooted radial gene tree analysis of the *dkk* genes among vertebrates, the zebrafish *dkk3* and *dkk3r* we cloned were homologous of the *dkk3* of other higher vertebrates. Using reverse transcription-polymerase chain reaction and whole-mount *in situ* hybridization, we demonstrated that both *dkk3* and *dkk3r* were maternally expressed. In addition, *dkk3* and *dkk3r* were ubiquitously expressed during 16 h post-fertilization (hpf). However, they were expressed in the head, somite and spinal cord at 24 hpf. Interestingly, while *dkk3* was particularly detected in the craniofacial neuron tissue after 24 hpf, *dkk3r* was restricted in craniofacial arch muscles and pancreas. These evidences suggested that *dkk3* and *dkk3r* shared the same expression patterns before 24 hpf, but displayed different patterns after 24 hpf. Thus, using zebrafish as our system model, it is suggested that the results, as noted above, may provide more insight into the molecular structures and expression patterns of the lower vertebrate *dkk3* genes.

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Program/Abstract # 266
B1 SOX coordinate cell specification with patterning and morphogenesis in the early zebrafish embryo
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The B1 SOX transcription factors SOX1/2/3/19 have been implicated in various processes of early embryogenesis. However,

their regulatory functions in stages from the blastula to early neurula remain largely unknown. In our present study, we systematically knocked down the B1 *sox* genes in zebrafish. Only the quadruple knockdown of the four B1*sox* genes *sox2/3/19a/19b* resulted in very severe developmental abnormalities, confirming that the B1*sox* genes are functionally redundant. Phenotypic analyses of the *sox2/3/19a/19b* quadruple knockdown embryos revealed that the B1 SOX proteins regulate the following distinct processes: (1) early dorsoventral patterning by controlling *bmp2b/7*; (2) gastrulation movements via the regulation of *pcdh18a/18b* and *wnt11*, a non-canonical Wnt ligand gene; (3) neural differentiation by regulating the *Hes*-class bHLH gene *her3* and the proneural-class bHLH genes *neurog1* (positively) and *ascl1a* (negatively), and regional transcription factor genes, e.g., *hesx1*, *zic1* and *rx3*; and (4) neural patterning by regulating signaling pathway genes, *cyp26a1* in RA signaling, *oep* in Nodal signaling, *shh*, and *mdkb*. Chromatin immunoprecipitation analysis of the *her3*, *hesx1*, *neurog1*, *pcdh18a* and *cyp26a1* genes further suggests a direct regulation of these genes by B1 SOX. These findings indicate that the B1 SOX proteins control a wide range of developmental regulators in the early embryo and suggest that the B1*sox* functions are central to coordinating cell fate specification with patterning and morphogenetic processes occurring in the early embryo.

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Program/Abstract # 267

Role of the dlx cis-regulatory elements I56i and I56ii in zebrafish GABAergic interneuron development

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The *Dlx* homeobox genes play a role in the differentiation, migration and survival of subpallial precursor cells that mainly give rise to GABAergic interneurons. They also regulate the *Gad* genes encoding the enzymes necessary for GABA synthesis. In mice, at least four *cis*-acting regulatory elements (CREs) control *Dlx* expression in the telencephalon and diencephalon: I12b and URE2 in the *Dlx1/2* bigene cluster; I56i and I56ii in the *Dlx5/6* cluster. We showed that I56i marks GABAergic progenitors in the ganglionic eminences and subtypes of GABAergic cortical interneurons in adult mice. Activity of I56ii is found in a subpopulation of GABAergic striatal projection neurons at E11.5-E13.5. To investigate whether similar *Dlx*-mediated pathways exist in zebrafish, we established lines of transgenic zebrafish with reporter constructs containing a 1.4kb *Dlx5a/6a* intergenic fragment (encompassing I56i and I56ii) or a 295 bp fragment of I56i. Preliminary data revealed that EGFP-positive cells in the two lines largely overlap, at least in some domains of the telencephalon. Co-expression of EGFP with various GABAergic interneuron markers was observed in cells of the telencephalon and diencephalon starting at 2 dpf. We are examining whether morpholino knockdown of the *dlx* genes causes reductions in CRE activity and interferes with GABAergic neuron development. These studies will help us better understand the functional involvement of the *dlx* genes in an evolutionarily conserved pathway controlling GABAergic interneuron differentiation. Supported by CIHR, NSERC, and the Department of Cellular and Molecular Medicine, University of Ottawa.

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Program/Abstract # 268

Swi/Snf chromatin remodeling complexes control zebrafish neural patterning and differentiation

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In eukaryotic nuclei Swi/Snf chromatin remodeling complexes control nucleosome occupancy, balancing the necessity of DNA packaging with that of access to DNA by regulatory factors. Swi/Snf targeting is controlled by subunit make up, which is regulated by cell type and differentiation state. Swi/Snf complexes broadly belong either to the BAF or PBAF families. PBAF complexes are distinguished by the inclusion of specific subunits, such as Arid2. Using a morpholino targeting the zebrafish *arid2* gene, we have investigated Arid2 mediated PBAF function during development. Neural tissues are particularly sensitive to Arid2 depletion. *arid2* morphants lack specific neuronal and sensory cell populations and have posteriorization of the anterior neural plate. While the signals responsible for neural plate anteriorization appear normal in the absence of Arid2, expression domains of markers of forebrain and midbrain identities are reduced at the end of gastrulation. The reduction in anterior neural fates contrasts with hindbrain and spinal regionalizations, which are anteriorly shifted but otherwise normal. Therefore, anteriorizing signals are either not received or improperly transduced in *arid2* morphants. In order to identify potential Arid2 transcriptional targets responsible for anterior neural fates we are using ChIP-Seq techniques. Our findings demonstrate that we can successfully identify cell type and developmental stage specific roles for Swi/Snf chromatin remodeling complexes using whole animal targeting of specific Swi/Snf subunits.

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Program/Abstract # 269

Fox1 and Fox4 regulate muscle-specific splicing in zebrafish and are required for cardiac and skeletal muscle functions

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Fox RNA binding proteins are important regulators of tissue-specific splicing in vertebrates. Using human exon array and zebrafish bioinformatic data, we have identified and validated numerous muscle-enriched exons with conserved Fox binding motifs in adjacent introns. We tested the function of Fox proteins using antisense-mediated knockdown in zebrafish embryos. Depletion of two muscle-enriched fox paralogs, Fox1 and Fox4, results in significant changes in splicing of 12 predicted target exons and uncovers both distinct and redundant roles for Fox1 and Fox4 in the regulation of alternative splicing. Furthermore, combined Fox1/Fox4 depletion induces specific and dramatic morphological defects. Despite a relatively normal overall appearance, Fox1/Fox4-depleted embryos exhibit ventricular hypotrophy, reduced heartbeat, and blood circulatory defects. Additionally, depleted embryos are nearly completely paralyzed, indicating that Fox proteins regulate genes that have a role in muscle contraction and/or motor neuron function rather than in skeletal muscle specification. Importantly, Fox-depleted embryos co-injected with fox mRNAs rescue splicing of predicted Fox-regulated exons and the cardiac, blood, and motility defects. Our findings indicate that Fox proteins are important regulators of muscle-specific splicing